TS013
Development of bioactive constructs through cell crosslinking for tissue regeneration

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Injectable systems are particularly attractive for a minimally invasive approach in tissue engineering applications. Many different methods for *in situ* crosslinking have been investigated however the ability of cells to promote hydrogel formation has not been fully explored. This study addressed the hypothesis that cells can promote crosslinking of chitosan microparticles forming a tridimensional network. Through covalent immobilization we were able to functionalize particles with specific antibodies, used to promote the attachment of cells and growth factors of interest. CD90 anti-human antibodies that are highly expressed by human adipose stem cells (hASCs) were successfully conjugated with chitosan microparticles. A tridimensional hydrogel was obtained by the assembly of the modified chitosan microparticles with hASCs and was stabilized by the crosslinks established by the entrapped cells. The degree of crosslinking of the structure could be regulated by the cell concentration in each construct. Moreover we believe that a combination of microparticles tailored with specific growth factors will increase the stability of the construct and promote cell differentiation. It is well established that platelets are an important source of autologous growth factors that can modulate cell growth and differentiation. In this study we propose antibody-conjugated particles as a method to select specific growth factors from the mixture obtained from platelet lysates. The obtained construct simultaneously provides support for stem cell growth as well localized and sustained presentation of factors to modulate cell differentiation. We intend to design a novel multifunctional injectable system that may be customized by combining particles with different growth factors for a specific application.

TS014
Liquified multilayered capsules incorporating microparticles for cell adhesion sites

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Polymeric multilayered capsules (PMCs) have found great applicability in various applications due to their versatile wall functions, capability to load active substances and unique permeability. PMCs are based on the sequential adsorption of polyelectrolytes by the layer-by-layer (LbL) technique, followed by the elimination or liquefaction of the template core. The principle of the strategy is to physically isolate a wide range of materials, including cells, proteins and/or therapeutic molecules, from the outside environment. This selective permeability is mediated by the LbL membrane, which allows the diffusion of nutrients, oxygen, waste products and metabolites, while avoiding the entrance of high molecular weight immune system components. However, when living cells are encapsulated, the existing methodologies still have to address a main issue related to the fact that most cells are anchorage-dependent and, thus, cannot grow in suspension. Therefore, although the liquified environment ensures the diffusion of essential molecules for cell survival, on the other hand liquified environments are deprived from cell adhesion sites. To overcome this main drawback, we hypothesized that liquified and flexible capsules combined with encapsulated microparticles to provide cell adhesion sites are a promising attempt. To test this hypothesis, hierarchical structures featuring (i) an external shell combining three polyelectrolytes, namely poly(L-lysine) (PLL), alginate (ALG) and chitosan (CHT) prepared by LbL, and (ii) incorporating surface functionalized poly(L-lactic) acid (PLLA) microparticles were developed. The construction of the multilayered structure by quartz-crystal microbalance with dissipation monitoring was monitored. Additionally, the mechanical performance of capsules was evaluated. Results show that the combined assembly of PLL, ALG and CHT resulted in a more resistant and thicker film with an exponential build-up growth regime compared to the assembly without PLL. The ability of the optimized capsules to support cell survival was assessed. L929 cells were encapsulated and cell viability and proliferation assays were performed. Results show that capsules containing PLLA microparticles revealed an enhanced metabolic activity, biocompatibility and proliferation. We believe that the developed approach will offer new possibilities to the existing bioencapsulation strategies. Different microparticles loaded with growth factors and other biomolecules of interest can be encapsulated in order to customize and control different cellular functions, such as differentiation of stem cells into the desired lineage, according to the target application field.

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